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Biochemical and functional characteristics of the photosynthetic apparatus in vegetative buds and mature needles of Norway spruce (*Picea abies* L. Karst.)

HRVOJE LEPEDUŠ*, VERA CESAR

Department of Biology, Faculty of Phylosophy, University J. J. Strossmayer,
L. Jägera 9, HR-31000 Osijek, Croatia

The biochemical characteristics and functioning of the photosynthetic apparatus in two developmental stages of spruce needles (vegetative buds and mature needles) were compared. Biochemical analysis included quantification of the photosynthetic pigments and immunodetection of the NAPDH-protochlorophyllide oxidoreductase (POR). Photosynthetic performance was monitored as the oxygen production at different light levels applied. Two POR isoforms were detected in buds while only one isoform was present in mature needles. The POR polypeptides in buds were expressed at different levels: the polypeptide of lower molecular mass had a level that was more enhanced than that of higher molecular mass. The most intensive POR signal was observed in mature needles. Concentrations of chlorophylls and carotenoids that were higher in mature needles than in vegetative buds were in accordance with observed POR expression. This reflected on the photosynthetic activity of the investigated organs. Although the chloroplasts in vegetative buds revealed the capability of photosynthesising, the compensation of respiration was not observed even at the highest light level (1100 $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$) applied. In contrast, mature needles ompensated for respiration successfully at low light level (30 $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$). Our study showed that chloroplasts in vegetative buds are photosynthetically competent, but limited by their molecular and biochemical background.

Key words: *Picea* abies, photosynthesis, oxygen production, respiration, NADPH-protochlorophyllide oxidoreductase, immunodetection, chlorophylls, carotenoids

Introduction

The development of photosynthetically active spruce needles starts with vegetative bud formation. This happens during the summer, after the elongation of the young shoots is complete (ROMBERGER 1966, BILKOVA et al. 1999). A detailed description of Norway spruce (*Picea abies* L. Karst.) vegetative bud structure is given by CESAR and BORNMAN (1996). It consists of an embryonic shoot subtended by a cup-like, cataphyll-bearing structure from which it is separated by a crown or nodal diaphragm. The bright green embryonic

* Corresponding author: Phone: (385 31) 211 400, Fax: (385 31) 212 514,
e-mail: hlepedus@yahoo.com

shoot contains the apical meristem, medulla or pith and the needle primordia. Using the primary chlorophyll fluorescence, LEPEDUŠ et al. (2003) showed that chloroplasts inside the vegetative buds are distributed in the medulla and needle primordia. An earlier study on the ultrastructure of chloroplasts in needle primordia done by SENSER et al. (1975) showed that these chloroplasts contain prolamellar bodies, a few thylakoids and starch grain. The weakly developed thylakoid system is maintained during the whole period of dormancy (LEPEDUŠ et al. 2001).

This study presents the continuation of the investigation into the molecular organisation and function of the photosystem II (PS II) in the vegetative buds and needles of spruce (LEPEDUŠ et al. 2004). A fully functional photosystem II was observed in both developmental stages, with the maximum quantum yield (Fv/Fm) values of 0.78 and 0.83 in vegetative buds and mature needles respectively. However, the light response curves revealed an effective quantum yield of photosystem II (ΔF/Fm') and relative electron transport rate (rel. ETR) values that were lower in vegetative buds than in needles. Molecular analysis of the light-harvesting complex of PS II (LHC II), cytochrome *b*-559 and the large subunit of Rubisco (LSU) revealed that the levels of all three investigated proteins were much lower in buds than in needles. This may account for the observed functional differences of photosystem II. The aim of this study was to correlate the expression of NADPH-protochlorophyllide oxidoreductase (POR), one of the key enzymes in chlorophyll biosynthesis, with the photosynthetic pigment content as well as to link this with the capability of buds and mature needles to compensate for dark respiration by oxygen production at different light levels applied.

Material and methods

The materials for study were vegetative buds and mature current-year needles collected from the middle crown of three Norway spruce (*P. abies* L. Karst.) trees. The sampling was done in April 2002. After removal of the cataphylls, the embryonic shoots of vegetative buds were excised and taken for analysis. Photosynthetic pigments were extracted with 80% acetone and determined spectrophotometrically according to LICHTENTHALER (1987). The amount of dry weight was determined by drying at 105 °C for 24 hours.

Photosynthetic activity was measured using a gas-phase Clark-type oxygen electrode (Hansatech, UK). Previously weighed plant material was placed in the chamber and the light response curves of oxygen evolution were performed by increasing the amount of light applied (30, 100, 200, 650 and 1100 $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$, respectively). The duration of each step was 20–30 minutes. Dark respiration was measured before and after the illumination as well as between each light step. The temperature inside the chamber was 20 °C.

The buds as well as the mature needles from all three trees were combined together making the samples for protein analysis. Plant material was powdered in liquid nitrogen and extracted with hot (80 °C) buffer containing 0.13 M Tris/HCl (pH 6.8), 16% (v/v) glycerol, 4.6% (w/v) sodium dodecyl sulphate (SDS) and 0.59% (v/v) dithiothreitol (DTT). For SDS polyacrylamide gel electrophoresis (SDS-PAGE), equal amount of total proteins (20 μg) of each sample was loaded onto the gel. Separated proteins were blotted onto the nitrocellulose membrane according to manufacturer's instructions. The immunodetection was performed using the appropriate antibodies developed to the NADPH-protochloro-

phyllide oxidoreductase (POR, provided by Dr. Jon Falk, Institute of Botany, University of Kiel, Germany). Anti-rabbit IgG linked with horseradish peroxidase was used as the secondary antibody. Visualization was done by chemiluminiscence using luminol as a substrate.

Statistical evaluation of the obtained data was performed by using t-test, modified for small sample (PAVLIĆ 1977).

Results and discussion

The photosynthetic performance of intact embryonic shoots and mature needles was measured as the oxygen production at different amounts of light applied. Results are expressed as the ratio between oxygen production and dark respiration (Fig. 1). Values of this ratio for the vegetative buds were 0.18, 0.30, 0.39, 0.47 and 0.52 at 30, 100, 200, 650 and 1100 $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$, respectively. The values for the mature needles were 1.18, 2.57, 3.24, 4.53 and 7.32 at 30, 100, 200, 650 and 1100 $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$, respectively. All values were shown to be significantly higher in mature needles than in vegetative buds, with P(t) values of below 1% or 0.1 (Tab. 1). Also, it can be seen that the values of the ratio be-

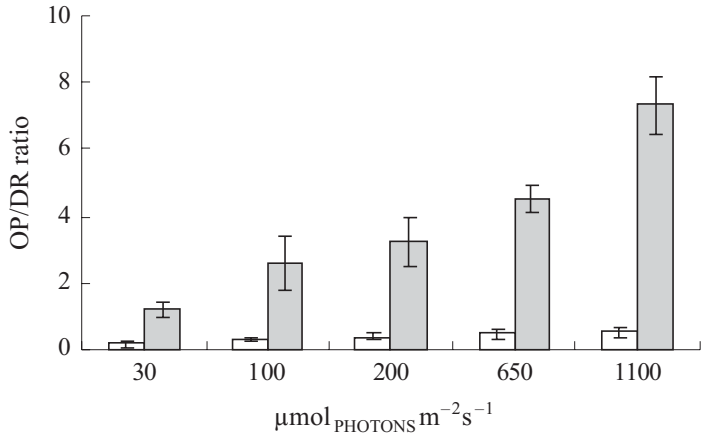

Fig. 1. Mean values of the oxygen production to dark respiration ratio (OP/DR) in vegetative buds (white bars) and mature needles (gray bars) of Norway spruce (*P. abies* L. Karst.) at different amounts of light applied (in $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$). Vertical bars indicate standard deviation.

Tab. 1. Statistical significances of the oxygen production to dark respiration ratio values between the mature needles and vegetative buds of Norway spruce (*P. abies* L. Karst.) at different light levels. P(t) – percent of similarity.

	Applied light levels (in $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$)				
	30	100	200	650	1100
Ratio needles *vs* buds	6.56	8.57	8.31	9.64	14.08
t	8.14	4.90	6.43	15.51	13.14
P(t)	<0.1%	<1%	<1%	<0.1%	<0.1%

tween oxygen production and dark respiration was about 6.5 times higher at low light level (30 $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$) while at high light level (1100 $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$) it was 14 times higher in needles than in buds (Tab. 1). The oxygen production in photosynthesis appears as the consequence of water oxidation. This process requires functional photosystem II, which utilizes the photons of absorbed light as the driving force (BRICKER and GHANOTAKIS 1996). A previous study of the photosystem II efficiency in spruce vegetative buds and needles (LEPEDUŠ et al. 2004) revealed the presence of functional photosystem II in both mature needles and vegetative buds. This would be in accordance with observed oxygen production in vegetative buds (Fig. 1). However, the inability to compensate for dark respiration, no matter how strong the light applied (Fig. 1), still remained the major characteristic of the photosynthetic apparatus in vegetative buds. In contrast, mature needles efficiently compensated for dark respiration at even a low light level (30 $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$).

The assembly of photosynthetically active thylakoid membranes during chloroplast differentiation is supposed to be preceded by the synchronized biosynthesis of the chloroplast pigments and the proteins of the photosynthetic apparatus (PLUMLEY and SCHMIDT 1995). The values of the total chlorophylls and total carotenoids are shown in Fig. 2. The concentration of the chlorophylls was 3.37 mg g^{-1} dry weight in mature needles and 1.12 mg g^{-1} dry weight in vegetative buds (Fig. 2A). The values of total carotenoid content were 1.11 and 0.44 mg g^{-1} dry weight in mature needles and vegetative buds, respectively (Fig. 2A). The ratio of chlorophyll *a* to chlorophyll *b* was 2.52 in mature needles and 3.05 in vegetative buds (Fig. 2B). The total chlorophyll to total carotenoid ratio was higher in mature needles (3.05) than in buds (2.52) (Fig. 2B). The observed differences in pigments content were proved to be statistically significant, with the exception of the chlorophyll *a* to chlorophyll *b* ratio (Tab. 2). The presented results are in accordance with our previous investigations on the photosynthetic pigments in vegetative buds and needles of spruce (LEPEDUŠ et al. 2001, 2003). We showed that a low level of chlorophylls and carotenoids was present in buds during the whole period of dormancy. The same level of pigments was maintained for

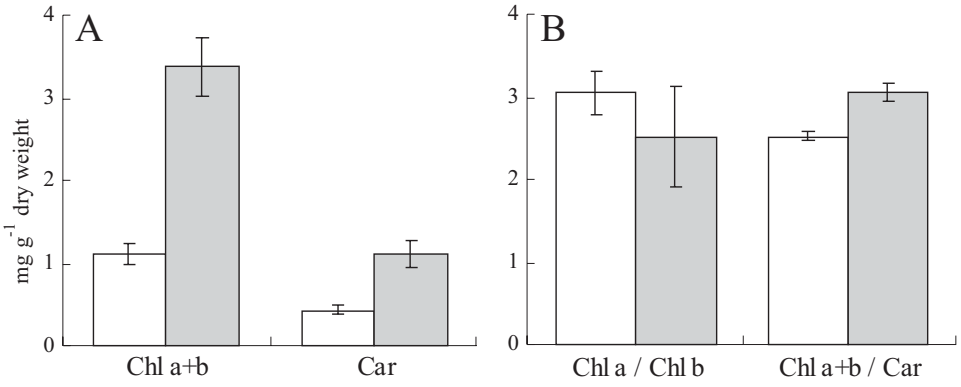

Fig. 2. Mean values of photosynthetic pigment content (A) and pigment ratios (B) in vegetative buds (white bars) and mature needles (gray bars) of Norway spruce (*P. abies* L. Karst.). Chl *a+b* – total chlorophylls, Car – total carotenoids, Chl *a* / Chl *b* – chlorophyll *a* to chlorophyll *b* ratio, Chl *a+b* / Car – total chlorophyls to total carotenoids ratio. Vertical bars indicate standard deviation.

Tab. 2. Statistical significances of the photosynthetic pigments values between mature needles and vegetative buds of Norway spruce (*P. abies* L. Karst.). Chl *a+b* – total chlorophylls, Car – total carotenoids, Chl *a* / Chl *b* – chlorophyll *a* to chlorophyll *b* ratio, Chl *a+b* / Car – total chlorophyls to total carotenoids ratio, P(t) – percent of similarity, NS – not significant.

	Chl *a+b*	Car	Chl *a* / Chl *b*	Chl *a+b* / Car
t	21,95	6,91	1,39	7,58
P(t)	<0.1%	<1%	NS	< 1%

a quite after the bud-bursting moment. The young needles needed a longer period of time (May to August) to reach the same level of photosynthetic pigment content as the needles formed in the previous season.

Chlorophyll biosynthesis involves a number of enzymes (VON WETTSTEIN et al. 1995, SUZUKI et al. 1997). Certain differences in the regulation of this process were pointed out when angiosperms and gymnosperms are compared. The most outstanding limitation in angiosperms is the reduction of protochlorophyllide to chlorophyllide *a*, a step that requires the presence of light. The formation of chlorophyllide was recognized as one of the control events in the assembly of the photosynthetic apparatus (EICHACKER et al. 1990). In contrast, gymnosperms have been known for a long time to be capable of chlorophyll biosynthesis in the dark (BOGDANOVIĆ 1973, BARTLET and DODGE 1980, WRISCHER et al. 1998). The expression of NADPH-protochlorophyllide oxidoreductase in vegetative buds and mature needles is shown in Fig. 3. The difference in isoform number was observed: two POR isoforms were present in the vegetative buds while only one was detected in the mature needles. The POR polypeptides in buds were expressed in different levels. The polypeptide of lower molecular mass had a much more enhanced level than that of higher molecular mass. The most intensive POR signal was observed in mature needles, indicating higher expression of POR in mature needles. Our observations (Fig. 3) correlate very well with these of FORREITER and APEL (1993). They investigated POR expression and localisation in cotyledons of dark- and light-grown mountain pine (*Pinus mugo* Turra, ssp. *Mugo*) seedlings. The authors reported the presence of two POR polypeptides in dark--grown seedlings. One of them was localised in the prolamellar bodies of etiochloroplasts and its abundance rapidly declined when the seedling were exposed to light. The second POR isoform was localised in the thylakoid membranes and was not affected upon the exposure of the dark-grown pine seedlings to light. The presence of two POR isoforms that

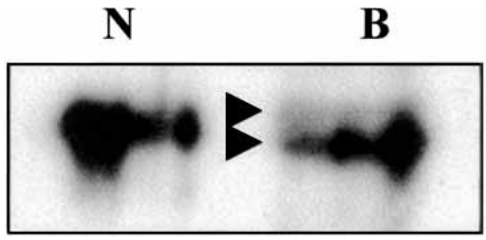

Fig. 3. Expression of NADPH-protochlorophyllide oxidoreductase (POR) in mature needles (N) and vegetative buds (B) of Norway spruce (*P. abies* L. Karst.) as analyzed by Western blots using specific antibodies.

we have detected in the embryonic shoots of vegetative buds could be explained in this manner, since the buds of spruce are covered with numerous cataphylls (ROMBERGER 1966, HEJNOWICZ and OBARSKA 1995) and in this way are deprived of the light.

It can be concluded that observed differences of NADPH-protochlorophyllide oxidoreductase expression between vegetative buds and mature needles (Fig. 3) might influence the biosynthesis of chlorophylls in these organs. Increased chlorophyll content in mature needles offered the possibility to harvest a sufficient amount of photons and compensate for dark respiration even at low irradiance (30 $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$). The failure of vegetative buds to compensate for dark respiration can be influenced in two ways. First, vegetative buds had three times higher dark respiration (3.87 $\mu mol\ O_2\ g^{-1}_{DRY\ WEIGHT}\ min^{-1}$) than mature needles (1.27 $\mu mol\ O_2\ g^{-1}_{DRY\ WEIGHT}\ min^{-1}$). The second reason should be sought in the molecular organisation of the electron transport chain in chloroplasts of vegetative buds. Our previous measurement of *in vivo* chlorophyll fluorescence kinetics (LEPEDUŠ et al. 2004) revealed that electron-transport chain in buds is saturated at 200 $\mu mol_{PHOTONS}\ m^{-2}s^{-1}$. This could be the main factor that downregulates photosystem II as well as the oxygen production in vegetative buds.

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